

## Supplemental Method

### Selecting potential GLP-1R interactors of interest

To narrow down the list of potential interactors to 3 interactors of interest for further validation and functional studies, we used four approaches: 1) identified in 2 cell lines; 2) located on the cell membrane according to the Uniprot database, 3) related to GPCR function according to literature (functional screening); and 4) associated with liganded GLP-1R (spectral counting). We estimated the protein abundance in unliganded and liganded states according to the exponentially modified protein abundance index (emPAI) score from Mascot searching, which is based on the number of observed peptides and the number of observable peptides per protein (1). The emPAI score of each GLP-1R interactor was normalized to the emPAI score of GLP-1R from the same run to estimate the relative abundance of the interactor binding to GLP-1R (Table S4). We compared potential interactors' abundance between unliganded and liganded states using a student's t-test, providing clues to find potential interactors that might be preferentially binding to liganded GLP-1R. Original emPAI scores of all potential GLP-1R interactors identified by AP-MS in CHO and MIN6 cells can be found in Table S6.

### Reference:

1. Ishihama Y, Oda Y, Tabata T, Sato T, Nagasu T, Rappsilber J, Mann M. (2005) Exponentially modified protein abundance index (emPAI) for estimation of absolute protein amount in proteomics by the number of sequenced peptides per protein. *Mol Cell Proteomics*. 4, 1265-1272